Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

Listing of Claims

1. (Currently amended) A pseudotyped-retrovirus-producing eukaryotic cell, comprising[:] a eukaryotic cell including nucleotide sequences operatively encoding components of a pseudotyped retrovirus, said nucleotide sequences comprising:

(a) a first nucleotide sequence operably encoding a retroviral Gag polypeptide;

(b) a second nucleotide sequence operably encoding a retroviral Pro polypeptide;

(c) a third nucleotide sequence operably encoding a retroviral Pol polypeptide; and

(d) a fourth nucleotide sequence operably encoding at least two different viral glycoproteins.

2. (Previously presented) The cell of claim 1, wherein said cell further comprises a fifth nucleotide sequence having a 5′ and a 3′ end, said fifth nucleotide sequence encoding a selected protein, said fifth nucleotide sequence operably linked at said 5′ end to a first retroviral long terminal repeat sequence and operably linked at said 3′ end to a second retroviral long terminal repeat sequence.

3. (Previously presented) The cell of claim 2, wherein said selected protein is a marker.

4. (Original) The cell of claim 3, wherein said marker is a fluorescent protein.

5. (Original) The cell of claim 1, wherein said two different viral glycoproteins are togaviral glycoproteins.

6. (Original) The cell of claim 5, wherein said togaviral glycoproteins are alphaviral glycoproteins.

7. (Original) The cell of claim 6, wherein said alphaviral glycoprotein is a Ross River alphaviral glycoprotein.

8. (Original) The cell of claim 1, wherein said eukaryotic cell is a mammalian cell.

9. (Original) The cell of claim 8, whererin said mammalian cell is a human cell.

10. (Original) The cell of claim 1, wherein said retroviral Gag, Pol and Pro polypeptides are comprised of Moloney murine leukemia Gag, Pro and Pol polypeptides.

11. (Original) The cell of claim 1, wherein said cell produces a pseudotyped retrovirus having a lipid bilayer, said viral glycoproteins disposed in said lipid bilayer.

12. (Original) The cell of claim 1, wherein said first, second, third and fourth nucleotide sequences are chromosomally-integrated.

13. (Withdrawn) A eukaryotic cell, comprising:

(a) a first nucleotide sequence encoding a retroviral Gag polypeptide;

(b) a second nucleotide sequence encoding a retroviral Pro

polypeptide;

(c) a third nucleotide sequence encoding a retroviral Pol polypeptide; and

(d) a fourth nucleotide sequence encoding a filoviral glycoprotein, said first, second, third and fourth nucleotide sequences being chromosomally-integrated, said cell stably producing pseudotyped retroviruses.

14. (Withdrawn) The cell of claim 13, wherein said cell further comprises a fifth nucleotide sequence having a 5′ end and a 3′ end, said fifth nucleotide sequence encoding a desired protein, said fifth nucleotide sequence operably linked at said 5′ end to a first retroviral long terminal repeat sequence and operably linked at said 3′ end to a second retroviral long terminal repeat sequence.

15. (Withdrawn) The cell of claim 13, wherein said filoviral glycoprotein is selected from the group consisting of Marburg virus glycoprotein and Ebola virus glycoprotein.

16. (Withdrawn) The cell of claim 13, wherein said retroviral Gag, Pro and Pol polypeptides are comprised of Moloney murine leukemia virus Gag, Pro and Pol polypeptides.

17. (Withdrawn) The cell of claim 13, wherein said cell produces pseudotyped retrovirus at a titer of at least about 4.5×10^4 transforming units/ml of supernatant.

18. (Withdrawn) A eukaryotic cell, comprising:

(a) a first nucleotide sequence encoding a retroviral Gag polypeptide;

(b) a second nucleotide sequence encoding a retroviral Pro polypeptide;

(c) a third nucleotide sequence encoding a retroviral Pol

polypeptide; and

(d) a fourth nucleotide sequence encoding a Marburg virus glycoprotein.

19. (Currently amended) A method of ~~forming~~ modifying a eukaryotic cell to prepare a pseudotyped-retrovirus-producing eukaryotic cell ~~for producing pseudotyped retroviruses~~, said method comprising:

transfecting a eukaryotic cell with a first nucleotide sequence operably encoding a retroviral Gag polypeptide, a second nucleotide sequence operably encoding a retroviral Pro polypeptide, a third nucleotide sequence operably encoding a retroviral Pol polypeptide and a fourth nucleotide sequence operably encoding at least two different viral glycoproteins.

20. (Original) The method of claim 19, wherein said first, second and third nucleotide sequences are operably linked to a promoter sequence.

21. (Original) The method of claim 19, wherein said viral glycoproteins are togaviral glycoproteins.

22. (Original) The method of claim 21, wherein said togaviral glycoproteins are alphaviral glycoproteins.

23. (Original) The method of claim 22, wherein said alphaviral glycoproteins are Ross River alphaviral glycoproteins.

24. (Canceled) The method of claim 19, wherein said first, second, third and fourth nucleotide sequences are chromosomally-integrated.

25. (Previously presented) The method of claim 19, wherein said cell further comprises a

fifth nucleotide sequence having a 5′ end and a 3′ end, said fifth nucleotide sequence encoding a selected protein, said fifth nucleotide sequence operably linked at said 5′end to a first retroviral long terminal repeat sequence and operably linked at said 3′ end to a second retroviral long terminal repeat sequence.

26. (Currently amended) A method of ~~forming~~ modifying a eukaryotic cell to prepare a pseudotyped-retrovirus-producing eukaryotic cell ~~for producing pseudotyped retroviruses~~, said method comprising:

(a) transfecting a eukaryotic cell with a vector including a first nucleotide sequence encoding a retroviral Gag polypeptide, a second nucleotide sequence encoding a retroviral Pro polypeptide and a third nucleotide sequence encoding a retroviral Pol polypeptide, said first, second and third nucleotide sequences operably linked to a first promoter sequence; and

(b) transfecting said cell with a fourth nucleotide sequence encoding at least two viral glycoproteins, said fourth nucleotide sequence operably linked to a second promoter sequence.

27. (Previously presented) The method of claim 26, said method further comprising transfecting said cell with a vector including a fifth nucleotide sequence having a 5′ and a 3′ end, said fifth nucleotide sequence encoding a selected protein, said fifth nucleotide sequence operably linked at said 5′ end to a first retroviral long terminal repeat sequence and operably linked at said 3′ end to a second retroviral long terminal repeat sequence.

28. (Previously presented) The method of claim 26, wherein said selected protein is a marker.

29. (Canceled) The method of claim 26, wherein said first, second, third and fourth nucleotide sequences are chromosomally-integrated.

30. (Withdrawn) A method of forming a eukaryotic cell for producing pseudotyped retroviruses, said method comprising:
(a) transfecting a eukaryotic cell with a first nucleotide sequence encoding a retroviral Gag polypeptide, a second nucleotide sequence encoding a retroviral Pro polypeptide, a third nucleotide sequence encoding a retroviral Pol polypeptide and a fourth nucleotide sequence encoding a filoviral glycoprotein, said first, second, third and fourth nucleotide sequences being chromosomally-integrated, said cell stably producing pseudotyped retroviruses.

31. (Withdrawn) The method of claim 30, wherein said filoviral glycoprotein is selected from the group consisting of Ebola virus glycoprotein and Marburg virus glycoprotein.

32. (Withdrawn) A method of forming a eukaryotic cell for producing pseudotyped retroviruses, said method comprising:

transfecting a eukaryotic cell with a first nucleotide sequence encoding a retroviral Gag polypeptide, a second nucleotide sequence encoding a retroviral Pro polypeptide, a third nucleotide sequence encoding a retroviral Pol polypeptide and a fourth nucleotide sequence encoding a Marburg virus glycoprotein.

33. (Original) A pseudotyped retrovirus, comprising:

(a) a retroviral capsid;

(b) a lipid bilayer; said lipid bilayer surrounding said retroviral capsid; and

(c) at least two different viral glycoproteins disposed in said lipid bilayer.

34. (Previously presented) The retrovirus of claim 33, said retrovirus further comprising a nucleotide sequence encoding a selected protein, said nucleotide sequence enclosed within said retroviral capsid.

35. (Original) The retrovirus of claim 33, wherein said viral glycoproteins are togaviral glycoproteins.

36. (Original) The retrovirus of claim 35, wherein said togaviral glycoproteins are alphaviral glycoproteins.

37. (Original) The retrovirus of claim 36, wherein said alphaviral glycoproteins are Ross River alphaviral glycoproteins.

38. (Original) The retrovirus of claim 33, wherein said retroviral capsid is comprised of a Moloney murine leukemia virus capsid.

39. (Withdrawn) A pseudotyped retrovirus, comprising:

(a) a retroviral capsid;

(b) a lipid bilayer; said lipid bilayer surrounding said retroviral capsid; and

(c) a Marburg virus glycoprotein disposed in said lipid bilayer.

40. (Currently amended) A method of introducing a selected nucleotide sequence into a cell~~, said method~~ comprising[:] transducing a cell ~~permissive for entry of a virus having at least two different viral glycoproteins in its lipid bilayer~~ with a pseudotyped retrovirus, said pseudotyped retrovirus comprising:

a selected nucleotide sequence;

a retroviral capsid;

a lipid bilayer surrounding said retroviral capsid; and

at least two different viral glycoproteins disposed in said lipid bilayer;

~~having a retroviral capsid;~~

~~a lipid bilayer, said lipid bilayer surrounding said retroviral capsid;~~

~~at least two different viral glycoproteins disposed in said lipid bilayer; and~~

~~a selected ribonucleotide sequence.~~

wherein said cell is permissive for entry of a pseudotyped retrovirus having at least two different viral glycoproteins in its lipid bilayer.

41. (Original) The method of claim 40, wherein said retroviral capsid is a Moloney murine leukemia virus capsid.

42. (Original) The method of claim 40, wherein said virus having at least two different glycoproteins in its lipid bilayer is a togavirus, and said at least two different viral glycoproteins are togaviral glycoproteins.

43. (Original) The method of claim 42, wherein said togavirus is an alphavirus and said togaviral glycoproteins are alphaviral glycoproteins.

44. (Withdrawn) A method of introducing a nucleotide sequence into a cell, said method comprising:

transducing a cell permissive for Marburg virus entry with a pseudotyped retrovirus having

a retroviral capsid;

a lipid bilayer; said lipid bilayer surrounding said retroviral capsid;

a Marburg virus glycoprotein disposed in said lipid bilayer; and

a desired ribonucleotide sequence.

45. (Withdrawn) A method of screening agents effective in blocking viral entry into a cell, said method comprising:

(a) treating a pseudotyped retrovirus with said agent, said pseudotyped retrovirus having a retroviral capsid;

a lipid bilayer, said lipid bilayer surrounding said retroviral capsid;

at least two different viral glycoproteins disposed in said lipid bilayer; and

a nucleotide sequence encoding a desired marker, said nucleotide sequence enclosed within said retroviral capsid;

(b) treating a cell permissive for entry of a virus having at least two different viral glycoproteins disposed in its lipid bilayer with said treated pseudotyped retrovirus; and

(c) identifying eukaryotic cells having the desired marker.

46. (Withdrawn) The method of claim 45, wherein said virus having at least two different viral glycoproteins disposed in its lipid bilayer is a togavirus and said two different viral glycoproteins are togaviral glycoproteins.

47. (Withdrawn) The method of claim 46, wherein said togavirus is an alphavirus and said togaviral glycoproteins are alphaviral glycoproteins.

48. (Withdrawn) The method of claim 45, wherein said agent is an immunological agent.

49. (Withdrawn) The method of claim 45, wherein said agent is a pharmacological agent.

50. (Withdrawn) A method of screening agents effective in blocking Marburg virus entry into a cell, said method comprising:

(a) treating a pseudotyped retrovirus with said agent, said pseudotyped retrovirus having a retroviral capsid;

a lipid bilayer, said lipid bilayer surrounding said retroviral capsid;

a Marburg virus glycoprotein disposed in said lipid bilayer; and

a nucleotide sequence encoding a desired marker, said nucleotide sequence enclosed within said retroviral capsid;

(b) treating a cell permissive for Marburg virus entry with said treated pseudotyped retrovirus; and

(c) identifying eukaryotic cells having the desired marker.

51. (Withdrawn) A method of screening agents effective in blocking viral entry into a cell, said method comprising:

(a) treating a cell permissive for entry of a virus having at least two different viral glycoproteins in its lipid bilayer with said agent;

(b) contacting said treated cell with a pseudotyped retrovirus having a retroviral capsid;

a lipid bilayer, said lipid bilayer surrounding said retroviral capsid;

at least two different viral glycoproteins disposed in said lipid bilayer;

a nucleotide sequence encoding a desired marker, said nucleotide sequence enclosed within said retroviral capsid; and

(c) identifying eukaryotic cells having the desired marker.

52. (Withdrawn) A method of screening agents effective in blocking viral entry into a cell, said method comprising:

(a) treating a cell permissive for entry of a Marburg virus with said agent;

(b) contacting said treated cell with a pseudotyped retrovirus having a retroviral capsid;

a lipid bilayer, said lipid bilayer surrounding said retroviral capsid;

a Marburg virus glycoprotein disposed in said lipid bilayer;

a nucleotide sequence encoding a desired marker, said nucleotide sequence enclosed within said retroviral capsid; and

(c) identifying eukaryotic cells having the desired marker.

53. (Currently amended) A kit for ~~forming a pseudotyped retrovirus~~ modifying a eukaryotic cell to prepare a pseudotyped-retrovirus-producing eukaryotic cell, said kit comprising:

(a) a first nucleotide sequence operably encoding a retroviral Gag polypeptide;

(b) a second nucleotide sequence operably encoding a retroviral Pro polypeptide;

(c) a third nucleotide sequence operably encoding a retroviral Pol polypeptide; and

(d) a fourth nucleotide sequence operably encoding at least two different viral glycoproteins; and

(e) means for transfecting a eukaryotic cell with said first, second, third, and fourth nucleotide sequences.

54. (Withdrawn) The method of claim 52, wherein said viral glycoproteins are togaviral glycoproteins.

55. (Withdrawn) A kit for forming a pseudotyped retrovirus, said kit comprising:

(a) a first nucleotide sequence encoding a retroviral Gag polypeptide;

(b) a second nucleotide sequence encoding a retroviral Pro polypeptide;

(c) a third nucleotide sequence encoding a retroviral Pol polypeptide; and

(d) a fourth nucleotide sequence encoding a Marburg virus glycoprotein.

56. (New) The method of claim 19, wherein the first, second, third, and fourth nucleotide sequences are provided on plasmid vectors.

57. (New) The method of claim 56, wherein the first, second, and third nucleotide sequences are contiguous on a single plasmid vector.

58. (New) The method of claim 57, wherein the fourth nucleotide sequence is on a different plasmid vector.